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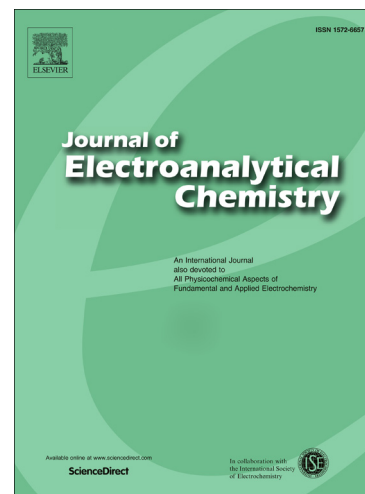
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Synthesis and electrochemical study of new 3-(hydroxyphenyl)benzo[f]coumarins

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Abstract

New hydroxyl substituted 3-arylbenzo[f]coumarins (compounds **6-10**) have been designed and synthesized. Their electrochemical redox mechanisms, and the influence of one or two hydroxyl groups, in different positions on the coumarin scaffold, was investigated by cyclic, differential pulse and square wave voltammetry, at a glassy carbon electrode, at different pHs, and a comparative study was performed. The structural information obtained enabled a better understanding of the structure/electrochemical relationship of hydroxyl substituted 3-arylbenzo[f]coumarins, compounds with important antioxidant properties.

Keywords: 3-(hydroxyphenyl)benzo[f]coumarins, oxidation, glassy carbon electrode, cyclic voltammetry, differential pulse voltammetry, square wave voltammetry.

1. Introduction

Phenolic compounds are bioactive substances widely distributed in the vegetable kingdom, containing one or more aromatic benzene rings with one or more hydroxyl groups and their properties are related to their chemical structure. Their importance is due to the broad biological and pharmacological activity, the role as antioxidants and implication in the prevention of pathologies such as cardiovascular and neurodegenerative diseases, cancer and inflammatory disorders, and their presence contribute to the colour, flavour and aroma of food [1-3].

Phenolic compounds mechanism of action as antioxidants is due to the ability of phenols to scavenge radicals by an electron transfer process in which a phenol is converted into a phenoxyl radical. Most phenolic compounds can be electrochemically oxidized due to the hydroxyl groups attached to the aromatic rings [4].

It is known that pH is one of the most significant factors determining the antioxidant activity of phenolic compounds. The dependence of the phenol derivatives oxidation potential on solution pH has been studied thoroughly for different classes of polyphenols [5-11], for the evaluation of the antioxidant capacity of several polyphenols and their mixtures [12], and for the understanding of their reaction mechanisms.

Resveratrol, a 3,4',5-trihydroxystilbene, produced by some spermatophytes species, such as vines, in response to external damage, is a natural polyphenolic compound extensively studied [13] due to anti-inflammatory, antioxidant, cardio-protective (vasodilator and platelet anti-aggregator), anticancer and enzymatic inhibitory properties [14,15].

Coumarins are a wide group of heterocyclic compounds present mainly in the vegetable kingdom, structurally constituted by the fusion of a pyrone with a benzene ring [16-18], with an important role in synthetic organic and medicinal chemistry [19]. Numerous

biological activities depend on the substitution pattern [20], offered by substitution and conjugation, leading to many synthetic analogues featuring a coumarin structural motif [21]. Coumarins are also described as antiviral, vasorelaxant, antioxidant, antimicrobial, anticancer, anti-inflammatory and enzymatic inhibitors [22-33], but limited data is available concerning their antioxidant activity [22, 30, 34-36].

In this paper a family of new compounds, in which the double ring nucleus of the stilbene, in its *trans* configuration, is “blocked” in the benzo[*f*]coumarin skeleton (naphthalene-coumarin hybrid), leading to naphthalene-coumarin-stilbene hybrid compounds, was synthesised, Scheme 1. Their electrochemical oxidation behaviour was investigated for a wide range of solution conditions, using a glassy carbon electrode and cyclic, differential and square wave voltammetry, at different pH, and the results will play a crucial role in understanding coumarin derivatives antioxidant activity.

2. Experimental

2.1 Materials and methods for the synthesis of 3-(hydroxyphenyl)benzo[*f*]coumarins (6-10)

The aim was to study and compare one derivative with a catechol group (3',4'-OH derivative) and another one presenting no contiguous OH groups (3',5'-OH derivative). Both precursors (2',5'-dimethoxy and 3',5'-dimethoxy derivatives) being commercially available on Aldrich. The 3',5'-OH derivative was chosen in order to maintain one of the positions of the other dihydroxy derivative (position 3').

Derivatives **6-10** were efficiently synthesized according to the protocol outlined, Scheme 2. Perkin condensation [19, 33, 37-41] of 2-hydroxy-1-naphthaldehyde with the corresponding arylacetic acids, using *N,N'*-dicyclohexylcarbodiimide (DCC) as dehydrating

agent, in DMSO, afforded the 3-(methoxyphenyl)benzo[*f*]coumarins **1-5**. Compounds **6-10** were synthesized starting from the respective methoxy derivatives **1-5** by hydrolysis reaction, using hydriodic acid 57%.

The melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus. ¹H- and ¹³C-NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in δ values, *J* in Hz). Mass spectra were obtained using a Hewlett Packard 5988A spectrometer. Elemental analyses were performed using a Perkin-Elmer 240B microanalyser and were within $\pm 0.4\%$ of calculated values in all cases. Silica gel (Merck 60, 230–00 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm).

2.2 General procedures for the synthesis of 3-(hydroxyphenyl)benzo[*f*]coumarins (**6-10**)

To a solution of the 2-hydroxy-1-naphthaldehyde (7.34 mmol) and the conveniently methoxy-substituted phenylacetic acid (9.18 mmol) in dimethyl sulfoxide (15 mL), DCC (11.46 mmol) was added and the mixture was heated in an oil-bath at 110 °C for 24 h. Triturate ice (100 mL) and acetic acid (10 mL) were added to the reaction mixture. After keeping it at room temperature for 2 h, the mixture was extracted with ether (3 x 25 mL). The organic layer was extracted with sodium bicarbonate solution (50 mL, 5%) and then water (20 mL). The solvent was dried with sodium sulfate and evaporated under vacuum. The residue was purified by FC (hexane/ethyl acetate 9:1) to give 3-(methoxyphenyl)benzo[*f*]coumarins (**1-5**).

To a solution of the corresponding 3-(methoxyphenyl)benzo[*f*]coumarin (**1-5**) (0.50 mmol) in acetic acid (5 mL) and acetic anhydride (5 mL) at 0 °C, hydriodic acid 57%

(10 mL) was added dropwise. The mixture was stirred, under reflux, for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by CH₃CN crystallization to give the corresponding 3-(hydroxyphenyl)benzo[f]coumarins (**6-10**) in yields between 60-67% in this last reaction, Scheme 2.

3-(4'-Hydroxyphenyl)benzo[f]coumarin (6) Yield 60%; mp 215-216 °C. ¹H-NMR (DMSO-*d*₆): 6.96 (m, 2H, H-3', H-5'), 7.51 (d, 1H, *J* = 9.0 Hz, H-10), 7.64 (m, 2H, H-6, H-7), 7.73 (m, 2H, H-2', H-6'), 7.96 (m, 2H, H-9, H-5), 8.32 (d, 1H, *J* = 8.5 Hz, H-8), 8.55 (s, 1H, H-4), 9.70 (s, 1H, OH). ¹³C-NMR (DMSO-*d*₆): 114.5, 117.3, 122.8, 126.6, 128.5, 128.7, 129.2, 129.5, 130.3, 130.9, 133.5, 136.4, 153.2, 158.4, 160.9. MS *m/z* (%): 414 (13), 288 (M⁺, 100), 260 (61), 231 (21), 202 (27), 130 (11), 84 (17), 66 (19). Anal. Calcd for C₁₉H₁₂O₃: C, 79.16; H, 4.20. Found: C, 79.18; H, 4.22.

3-(3'-Hydroxyphenyl)benzo[f]coumarin (7) Yield 63%; mp 208-209 °C. ¹H-NMR (DMSO-*d*₆): 6.83 (m, 1H, H-4'), 7.27 (m, 3H, H-2', H-5', H-6'), 7.56 (d, 1H, *J* = 9.0 Hz H-10), 7.65 (m, 2H, H-6, H-7), 8.02 (d, 1H, *J* = 8.2 Hz, H-5), 8.15 (d, 1H, *J* = 9.0 Hz, H-9), 8.66 (d, 1H, *J*=8.2 Hz, H-8), 8.88 (s, 1H, H-4), 9.66 (s, 1H, OH). ¹³C-NMR (DMSO-*d*₆): 113.9, 116.1, 116.2, 116.8, 120.0, 123.1, 126.6, 126.6, 128.7, 129.2, 129.3, 129.7, 130.4, 133.4, 136.4, 136.8, 152.9, 157.4, 160.2. MS *m/z* (%): 289 (22), 288 (M⁺, 100), 260 (80), 231 (17), 202 (26), 130 (12). Anal. Calcd for C₁₉H₁₂O₃: C, 79.16; H, 4.20. Found: C, 79.15; H, 4.21.

3-(2'-Hydroxyphenyl)benzo[f]coumarin (8) Yield 65%; mp 216-217 °C. ¹H-NMR (DMSO-*d*₆): 6.90 (m, 2H, H-3', H-5'), 7.30 (m, 2H, H-4', H-6'), 7.61 (d, 1H, *J* = 9.0 Hz, H-10), 7.64 (m, 2H, H-6, H-7), 8.06 (d, 1H, *J* = 7.2 Hz, H-5), 8.19 (d, 1H, *J* = 9.0 Hz, H-9), 8.60 (d, 1H, *J*=8.2 Hz, H-8), 8.55 (s, 1H, H-4), 9.61 (s, 1H, OH). ¹³C-NMR (DMSO-*d*₆): 111.7, 113.9,

116.8, 120.0, 121.0, 123.8, 124.5, 126.4, 128.2, 128.9, 129.2, 130.2, 130.2, 131.1, 132.4, 137.2, 153.0, 156.2, 160.1. MS m/z (%): 288 (M^+ , 100), 271 (16), 260 (44), 231 (47), 202 (30), 101 (13). Anal. Calcd for $C_{19}H_{12}O_3$: C, 79.16; H, 4.20. Found: C, 79.14; H, 4.19.

3-(3',4'-Dihydroxyphenyl)benzo[f]coumarin (9) Yield 67%; mp 236-237 °C. 1H -NMR (DMSO- d_6): 6.83 (d, 1H, J = 8.2 Hz, H-5'), 7.20 (d, 1H, J = 9.0 Hz, H-10), 7.35 (m, 2H, H-5, H-7), 7.47 (s, 1H, H-2'), 7.67 (m, 1H, H-6), 8.04 (d, 1H, J = 8.2 Hz, H-6'), 8.13 (d, 1H, J = 9.0 Hz, H-9), 8.70 (d, 1H, J = 8.5 Hz, H-8), 8.82 (s, 1H, H-4), 9.05 (s, 1H, OH), 9.23 (s, 1H, OH). ^{13}C -NMR (DMSO- d_6): 111.0, 112.7, 114.4, 117.0, 123.2, 123.8, 126.5, 126.9, 128.4, 128.6, 129.3, 130.6, 132.6, 134.8, 149.6, 150.7, 153.6, 156.1, 160.8. MS m/z (%): 304 (M^+ , 100), 276 (71), 202 (14), 101 (14). Anal. Calcd for $C_{19}H_{12}O_4$: C, 74.99; H, 3.97. Found: C, 74.97; H, 3.95.

3-(3',5'-Dihydroxyphenyl)benzo[f]coumarin (10) Yield 65%; mp 244-245 °C. 1H -NMR (DMSO- d_6): 6.31 (s, 1H, H-4'), 6.71 (s, 2H, H-2', H-6'), 7.59 (d, 1H, J = 9.0 Hz, H-10), 7.64 (m, 2H, H-6, H-7), 8.06 (d, 1H, J = 8.1 Hz, H-5), 8.18 (d, 1H, J = 9.0 Hz, H-9), 8.71 (d, 1H, J = 8.1 Hz, H-8), 8.87 (s, 1H, H-4), 9.38 (s, 2H, OH). ^{13}C -NMR (DMSO- d_6): 101.5, 103.4, 107.5, 113.9, 116.8, 123.1, 126.5, 126.9, 128.6, 129.2, 129.3, 130.4, 133.3, 136.5, 136.8, 152.9, 158.5, 158.7, 160.0. MS m/z (%): 304 (M^+ , 100), 276 (78), 189 (10), 152 (10), 138 (10). Anal. Calcd for $C_{19}H_{12}O_4$: C, 74.99; H, 3.97. Found: C, 74.97; H, 3.95.

2.3 Voltammetric conditions

The 0.1 M ionic strength supporting electrolyte solutions: pH 2.0 KCl/HCl, pH 3.4-5.4 acetate buffer, pH 6.1-8.0 phosphate buffer, pH 9.2-10.5 ammonia buffer, and pH

12.0 NaOH/KCl, were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity $\leq 10 \mu\text{S/cm}$) [42]. Experiments were carried out at room temperature ($25 \pm 1^\circ\text{C}$) and in the presence of dissolved oxygen.

The pH measurements were carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature ($25 \pm 1^\circ\text{C}$) and microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pipettes (Rainin Instrument Co. Inc., Woburn, USA).

Voltammetric experiments were carried out using an Autolab PGstat 10 running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Glassy carbon electrode (GCE, $d = 1.5 \text{ mm}$) was the working electrode, Pt wire the counter electrode and the Ag/AgCl (3 M KCl) reference electrode. Measurements were carried out using a three-electrode system in a 3 mL one-compartment electrochemical cell (Echem Electrode Kit/ref. ET014 and ET080-12, eDAQ Products, Poland).

The experimental conditions for CV were scan rate 50 mV s^{-1} . For differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1} . For square wave (SW) voltammetry were: pulse of 50 mV, frequency of 10 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 20 mV s^{-1} .

The GCE was polished using diamond particles of $3 \mu\text{m}$ (Kemet, UK) before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and voltammograms were recorded until a steady state baseline voltammograms were obtained. This procedure ensured very reproducible experimental results.

3. Results

A series of new compounds were synthesized with a basic skeleton of a naphthalene group at positions C5 and C6 and a phenyl group at position C3 of hydroxy-substituted coumarin ring (compounds **6-10**). The electrochemical oxidation of the hydroxylated 3-arylbenzo[f]coumarins, using CV, DP and SW voltammetry, at a GCE in a pH range of 1.25 to 12.3, was investigated. The results, concerning the number and position of hydroxylated substituents on the ring at position C3, were compared and the oxidation mechanisms proposed.

3.1 Cyclic voltammetry

CVs of compounds **6**, **7** and **8**, that have the same number of hydroxylated substituents, were carried out in the pH range between 1.3 and 12.3, at different concentrations, and the results compared.

CV in 20 μ M of compound **6**, in acetate buffer pH 5.3, showed an irreversible oxidation peak P₁, at $E_{p1} = + 0.705$ V, due to phenol oxidation. The oxidation of compound **6** gives rise to the formation of a reversible phenol oxidation product that corresponds to a catechol moiety, peak P_{2c}, at $E_{p2c} = + 0.305$ V, and peak P_{2a}, at $E_{p2a} = + 0.330$ V. The value of $|E_p - E_{p/2}| \sim 30$ mV indicates a reversible process with two electron transfer. Due to the occupancy of compound **6** *para* position it is not possible the phenol oxidation to give rise to the formation of a hydroquinone.

In the case of compounds **7** and **8**, both *ortho* and *para* positions are unrestricted (in the case of compound **7**, both *ortho* positions). Therefore, in acetate buffer pH 5.3,

compounds **7** and **8** phenol oxidation process gives rise to two oxidation products, due to the formation of a catechol and a hydroquinone.

Compound **7** irreversible phenol oxidation potential P_1 , at $E_{p1} = + 0.828$ V, $|E_p - E_{p/2}| = 0.045$ V, corresponds to one electron transfer reaction. The reversible phenol oxidation products, peaks P_{2a}/P_{2c} , at $E_{p2c} = + 0.356$ V and $E_{p2a} = + 0.388$ V, with $|E_p - E_{p/2}| = 0.031$ V, and peaks P_{3a}/P_{3c} , at $E_{p3c} = + 0.220$ V and $E_{p3a} = + 0.243$ V, with $|E_p - E_{p/2}| = 0.030$ V, occurred in a pH-dependent two electron transfer reaction, Fig. 1.

The strong adsorption of compound **7** phenol oxidation products at the surface of the GCE was demonstrated when, after several potential scans in the solution, the electrode was rinsed with a jet of deionized water and transferred to the supporting electrolyte, where the CV showed the reversible peaks P_2 and P_3 , and the current remained constant in successive scans, Fig. 2.

In compound **8** the peak potentials were very similar to those of compound **7**, and the two phenol oxidation product peaks are due to a catechol and hydroquinone moiety. Compound **8** irreversible phenol oxidation peak P_1 , at $E_{p1} = + 0.780$ V, $|E_p - E_{p/2}| = 0.045$ V, corresponds to one electron transfer. The reversible phenol oxidation product peaks P_{2a}/P_{2c} , at $E_{p2c} = + 0.396$ V and at $E_{p2a} = + 0.445$ V, $|E_p - E_{p/2}| = 0.038$ V, and peaks P_{3a}/P_{3c} , at $E_{p3c} = + 0.217$ V and at $E_{p3a} = + 0.244$ V, $|E_p - E_{p/2}| = 0.033$ V, occur in a pH-dependent two electron transfer reaction. Increasing the number of scans peaks P_{2a}/P_{2c} current decreased and P_{3a}/P_{3c} current increased due to the steric hindrance effect at the compound **8** *ortho*-hydroxyl group.

Compound **9** has a catechol group on its aromatic ring, whereby the oxidation potential expected is much lower than in the case of the phenol moiety. CVs at three different pHs: 3.5, 4.4 and 5.3, Fig. 3, showed a pH-dependent reversible oxidation peak P_1 ,

at $E_{p1a} = + 0.347$ V and $E_{p1c} = + 0.335$ V, of the two electron and two proton transfer oxidation of the catechol moiety.

Compound **10** presents a resorcinol group in the aromatic ring and the oxidation of each hydroxyl group is independent, and two irreversible oxidation peaks, P_1 , at $E_{p1a} = + 0.815$ V, and P_2 , at $E_{p2a} = + 0.998$ V, as expected in the oxidation of phenols, were observed, and the compound **10** reversible oxidation products present hydroquinone or catechol moieties.

3.2 Differential Pulse Voltammetry

CV showed that the oxidation of all compounds **6-10** was pH –dependent. The effect of pH on the oxidation of this novel coumarin-resveratrol hybrid compounds, peak P_1 , was deeply investigated by DP voltammetry, in different supporting electrolytes for a wide pH range. A shift on the oxidation potential of peak P_1 to more negative values with increasing pH, and a linear dependence with a slope of ~ 59 mV per pH unit was always observed, indicating that the oxidation of this novel coumarin-resveratrol hybrid compounds involved the same number of electrons and protons, Table 1.

The oxidation potential of peak P_1 of compound **6**, with a hydroxyl at the *para* position of the coumarin, and of compound **8**, with the hydroxyl in position *ortho* to the coumarin, are very similar at all pHs studied, whereas the oxidation potential of peak P_1 of compound **7** is higher, Fig. 4.

The oxidation potential of compound **9** peak P_1 , at $E_{P1} = + 0.160$ V, width at half height, $W_{1/2} = + 0.055$ V, in phosphate buffer pH 7.1, corresponded to the oxidation of the catechol moiety, Fig. 5B. The peak P_1 oxidation current, I_{p1} , has a maximum in acetate buffer

pH 4.4, Fig. 5B, and the slope of ~ 59 mV per pH unit indicated an oxidation process with the transfer of two electrons and two protons, Table 1.

The oxidation potentials of compound **10**, with two hydroxyls almost equivalent, are very close to peaks P_1 and P_2 , Fig. 6. An experiment by CV reversing the scan immediately after P_1 showed the occurrence of P_3 . Comparing the first DP voltammograms for compounds **7** and **10**, in acetate buffer pH 4.4, was found that compound **7** peak P_1 potential was similar to compound **10** peak P_2 potential. This explains the disappearance of compound **10** peak P_1 in the second scan, as the oxidation of one hydroxyl occurred, to form the corresponding oxidation product. The other hydroxyl group in a similar position to compound **7** has the oxidation potential peak P_2 . The effect of pH on compound **10** oxidation potential of peaks P_1 and P_2 , Fig. 7, was investigated. For $\text{pH} < 3$ the oxidation reactions are both pH-independent. For $\text{pH} > 3$ the oxidation reactions are both pH-dependent and the slope of ~ 59 mV per pH unit indicated a two electron and two proton transfers, Table 1.

DP voltammograms in different supporting electrolytes for a wide pH range, Table 1, of the oxidation products of all compounds **6-10** were also investigated and the oxidation is pH-dependent and always involved the transfer of two electrons and two protons. The oxidation product of compound **6** is a catechol group, and of compounds **7**, **8** and **10** are catechol and hydroquinone groups.

3.4 Square wave voltammetry

The advantages of SW voltammetry are greater speed of analysis, lower consumption of electroactive species in relation to DP voltammetry, and reduced problems with blocking of the electrode surface. A great advantage of the square-wave method is the possibility to see during one scan if the electron transfer reaction is reversible or not. Since the current is

sampled in both the positive and the negative-going pulses, peaks corresponding to the oxidation or reduction of the electroactive species at the electrode surface are obtained in the same experiment.

SW voltammetry of the monohydroxylated compounds confirmed the irreversible oxidation processes of compounds **6**, **7**, **8**, and of the dihydroxy compound **10**.

SW voltammograms of 50 μ M compound **8**, in pH 4.4 acetate buffer, showed in the first scan irreversible oxidation peak P_1 , at $E_{P1} = + 0.765$ V. In the subsequent scans, without cleaning the surface of the GCE, compound **8** reversible oxidations products peak P_2 , at $E_{P2} = + 0.464$ V, and peak P_3 , at $E_{P3} = + 0.230$ V, confirmed the oxidation of the catechol and hydroquinone groups, Fig. 8.

SW voltammograms of compound **9**, in acetate buffer pH 5.3, showed a single reversible peak P_1 , strongly adsorbed on the surface the GCE, Fig. 9.

4. Discussion

The new 3-(hydroxyphenyl)benzo[f]coumarins (compounds **1-10**) were efficiently synthesized, characterized, and the hydroxyl derivatives (**6-10**) were investigated for their antioxidant properties. The electrochemistry of this selected series of synthesized coumarin-naphthalene-stilbene hybrids (**6-10**) showed that the difference in the position of the hydroxyl substituents have only a slightly effect upon the electroactivity of the selected coumarins. However, differences in the number of the substituents on similar structures lead to specific differences in their voltammetric behaviour. A strong adsorption of the oxidation products of compounds **6-8** on the GCE surface was also observed.

The synthesized compounds **6-8** possess in common one hydroxyl group on *para* (compound **6**), *meta* (compound **7**) and *ortho* (compound **8**) positions of the 3-aryl ring.

Compounds **9** and **10** presented two hydroxyl groups in their structure, and compound **9** is a catechol derivative. The phenol group is irreversibly oxidised in compounds **6**, **7**, **8**, and **10**, and the catechol group is reversibly oxidised in compound **9**. The oxidation products of the coumarin-naphthalene-stilbene hybrids (**6-10**) are reversible and corresponded always to the formation of a catechol or hydroquinone moieties. All the compounds have in common the coumarin nucleus, which did not interfere in the different oxidation mechanisms. Based on the voltammetric research by CV, DP and SW voltammetry the reaction mechanisms for the oxidation of the newly synthesized 3-(hydroxyphenyl)benzo[*f*]coumarins were proposed.

The monohydroxylated compounds **6-8**, irreversible pH-dependent oxidation process, occurs with one electron and one proton transfer, following the phenol oxidation mechanism [43]. Compound **6**, with the *para* position to the hydroxyl group occupied, formed a single oxidation product corresponding to a catechol moiety, Scheme 3. Compounds **7** and **8** oxidation products corresponded to a catechol and a hydroquinone moiety, Scheme 4.

The dihydroxy compounds **9** and **10** undergo different oxidation mechanisms. Compound **9** reversible oxidation occurs at the catechol group without the formation of oxidation products, Scheme 5. Compound **10** first irreversible oxidation of one hydroxyl group is followed by the irreversible oxidation of other hydroxyl group, and each occurred with one electron and one proton transfer, Scheme 5. The oxidation Compound **10**, with two hydroxyl groups and the *para* position free, enabled the formation of two oxidation products, a hydroquinone and a catechol group that are oxidised each in two electrons and two protons transfer.

5. Conclusions

The synthesis of ten new 3-arylbenzo[f]coumarins (compounds **1-10**) was carried out in an efficient, direct and versatile way using a Perkin reaction as key step. The ether derivatives (compounds **1-5**) were hydroxylated, with good yields, giving the corresponding hydroxyl substituted 3-arylbenzo[f]coumarins (compounds **6-10**) that were pH-dependent electrochemically oxidised. Electrochemistry showed that all these novel coumarins are oxidized at relatively low potentials, and the phenol group oxidation is irreversible, except for compound **9** with a catechol in its structure. The oxidation products are reversible electroactive catechol or hydroquinone moieties. The oxidation mechanisms of this new series of hydroxyl substituted 3-arylbenzo[f]coumarins are proposed, and clearly showed their good antioxidant properties enabling pharmacological applications.

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Table 1. DP voltammetric data for compounds **6-10**.

	Peak P ₁			Peak P ₂			Peak P ₃			Peak P ₄		
	E_p vs. pH	e^-	H^+	E_p vs. pH	e^-	H^+	E_p vs. pH	e^-	H^+	E_p vs. pH	e^-	H^+
6	$E_p = 0.95-0.060$ pH	1	1	$E_p = 0.63-0.059$ pH	2	2	—	—	—	—	—	—
7	$E_p = 1.09-0.062$ pH	1	1	$E_p = 0.63-0.059$ pH	2	2	$E_p = 0.48-0.059$ pH	2	2	—	—	—
8	$E_p = 0.92-0.058$ pH	1	1	$E_p = 0.70-0.059$ pH	2	2	$E_p = 0.48-0.058$ pH	2	2	—	—	—
9	$E_p = 0.55-0.059$ pH	2	2	—	—	—	—	—	—	—	—	—
10	$E_p = 0.80-0.059$ pH	1	1	$E_p = 0.97-0.059$ pH	1	1	$E_p = 0.34-0.059$ pH	2	2	$E_p = 0.44-0.060$ pH	2	2

Figures and Schemes

Scheme 1. Synthesized compounds **6-10** chemical structure.

Scheme 2. Experimental conditions: (i) DCC, DMSO, 110 °C, 24 h; (ii) HI 57%, AcOH, Ac₂O, reflux, 3 h.

Scheme 3. Oxidation mechanism proposed for compound **6**.

Scheme 4. Oxidation mechanism proposed for compounds **7** and **8**.

Scheme 5. Oxidation mechanism proposed for compounds **9** and **10**.

Fig. 1. CV in 10 μ M compound **7**, in acetate buffer pH 4.4, at GCE: (—) first, (---) fifth and (···) tenth scan. Scan rate 50 mV s⁻¹.

Fig. 2. CV of compound **7** adsorbed at the GCE surface, in acetate buffer pH 4.4: (···) first, (—) fifth and (---) tenth scan after transfer to the buffer solution. Scan rate 50 mV s⁻¹.

Fig. 3. CV in 10 μ M compound **9**, first scan, in acetate buffer, at GCE: (---) pH 3.5, (—) pH 4.4 and (···) pH 5.3. Scan rate 50 mV s⁻¹.

Fig. 4. Plot of E_{p1a} vs. pH of compounds **6** (●), **7** (■) and **8** (★).

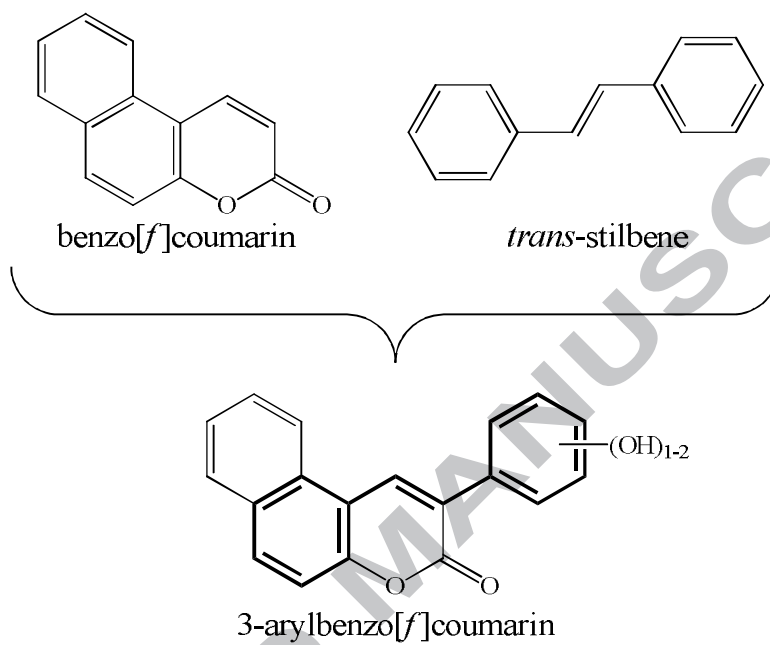
Fig. 5. (A) 3D plot of DP voltammograms baseline corrected in compound **9** vs. pH;
(B) Plot of E_{p1a} (●), and I_{p1a} (■) vs. pH.

Fig. 6. DP voltammogram in 50 μ M compound **10**, in acetate buffer pH 3.5: (—) first, (•••) second and (---) tenth scan. Scan rate 5 mV s⁻¹.

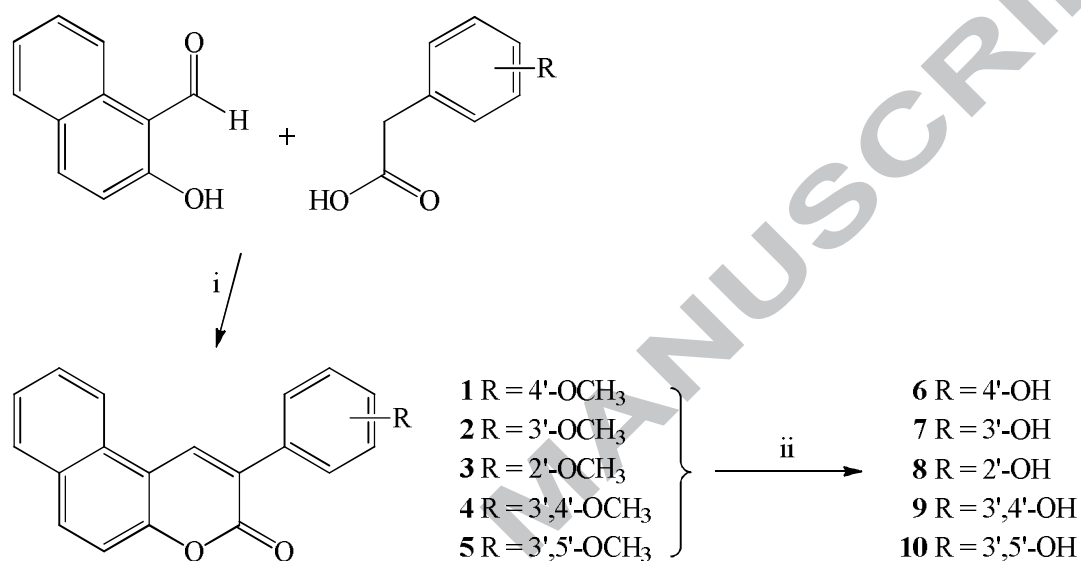
Fig. 7. Plot of E_{p1a} (●) and E_{p2a} (■) vs. pH of compound **10**.

Fig. 8. SW voltammograms in 50 μ M compound **8**, in acetate buffer pH 4.4: (A) first and (B) third scan. I_t – total current, I_f – direct current and I_b – forward current; $f = 25$ Hz, $\Delta E = 2$ mV, $v_{\text{eff}} = 50$ mV s⁻¹.

Fig. 9. SW voltammograms in 10 μ M compound **9** third scan, in acetate buffer at pH 5.3: I_t – total current, I_f – direct current and I_b – forward current; $f = 25$ Hz, $\Delta E = 2$ mV, $v_{\text{eff}} = 50$ mV s⁻¹.

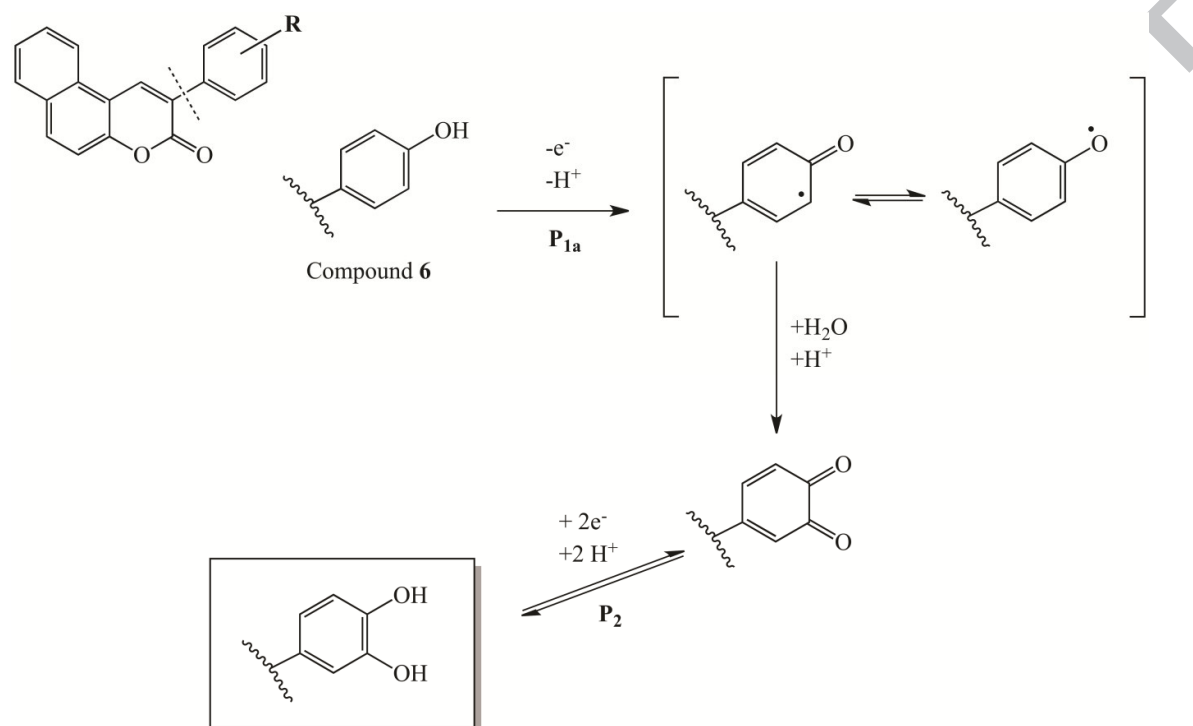


Scheme 1. Synthesized compounds **6-10** chemical structure.

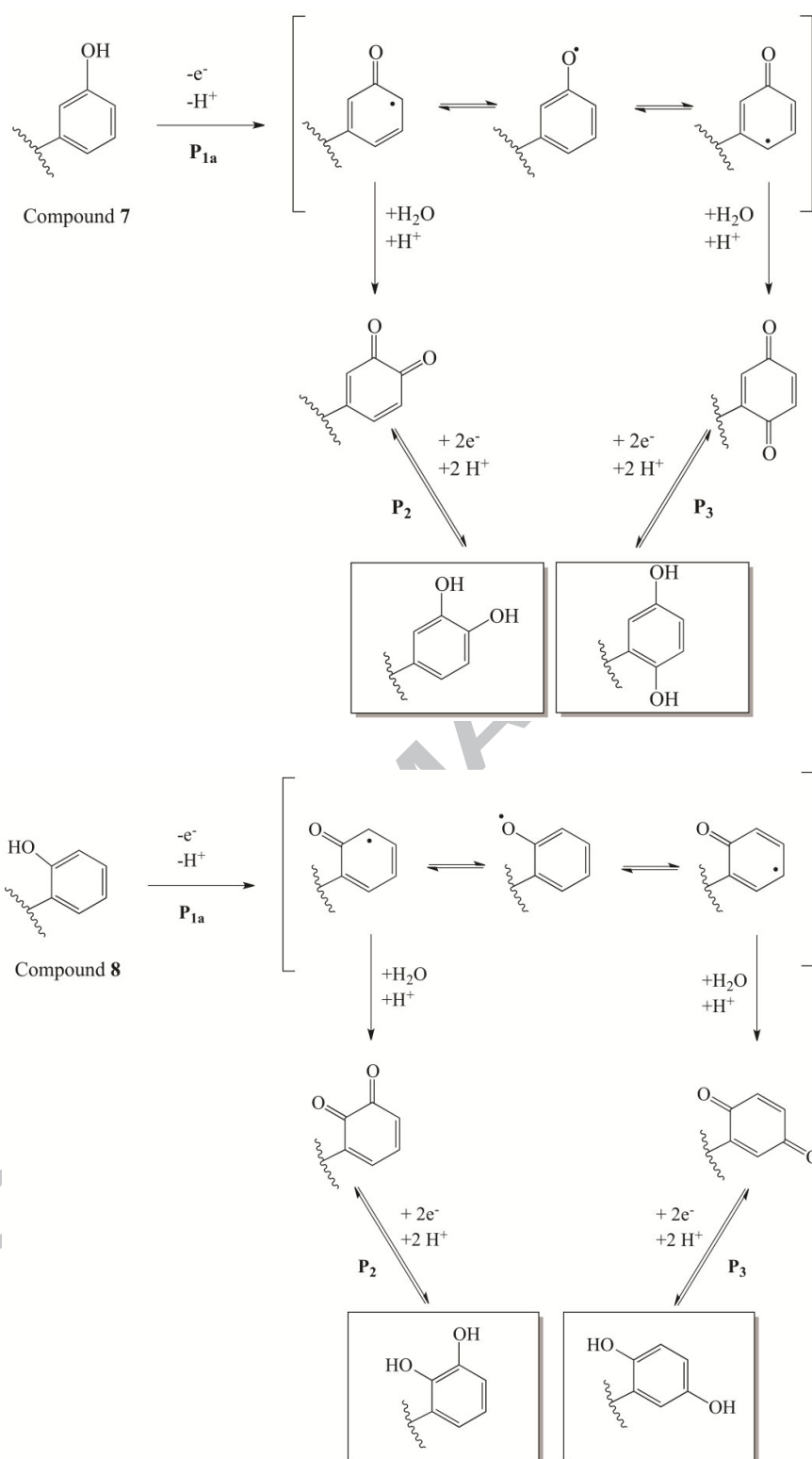


Scheme 2. Experimental conditions: (i) DCC, DMSO, 110 °C, 24 h;

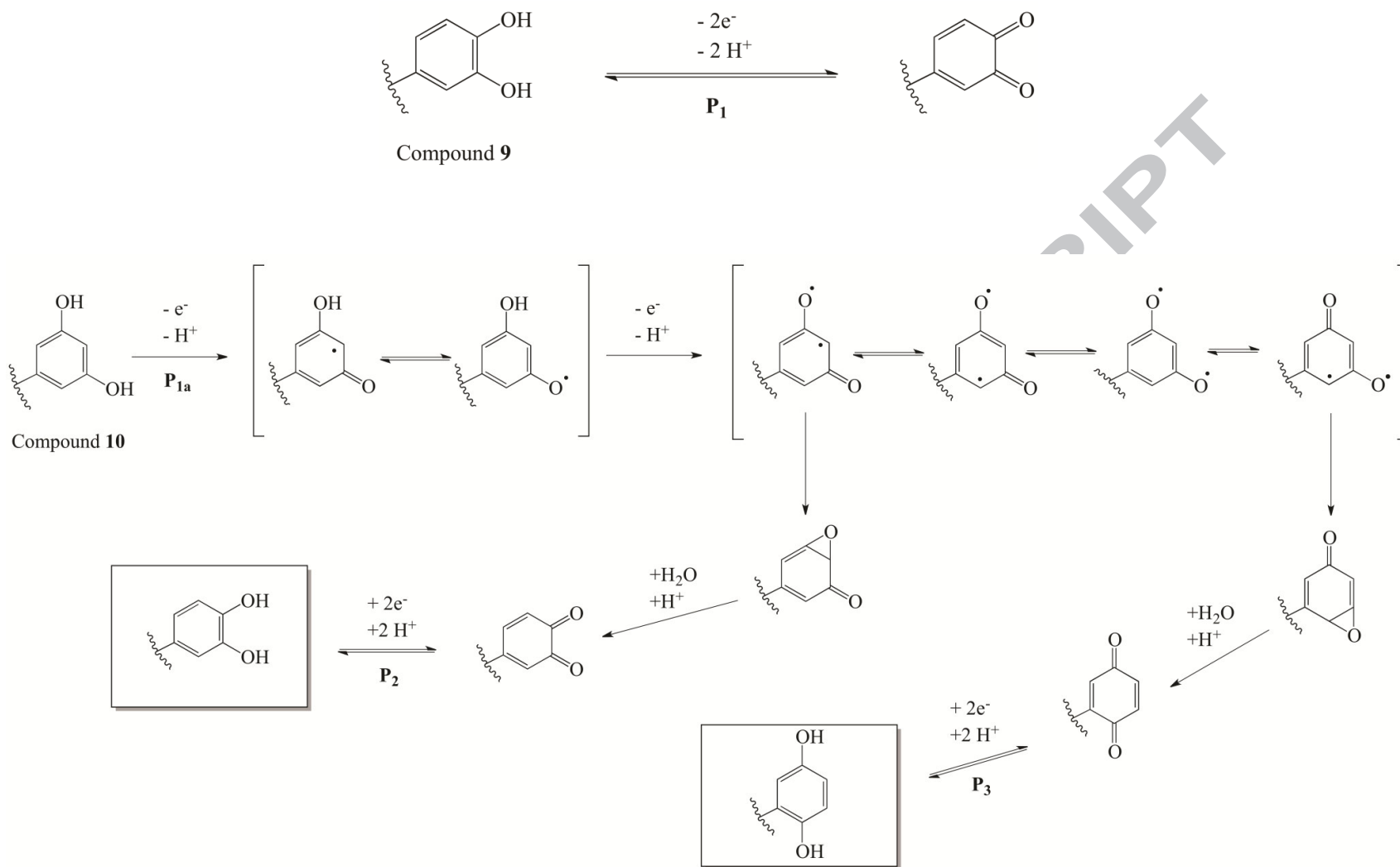
(ii) HI 57%, AcOH, Ac₂O, reflux, 3 h.



Scheme 3. Oxidation mechanism proposed for compound 6.



Scheme 4. Oxidation mechanism proposed for compounds **7** and **8**.



Scheme 5. Oxidation mechanism proposed for compounds 9 and 10.

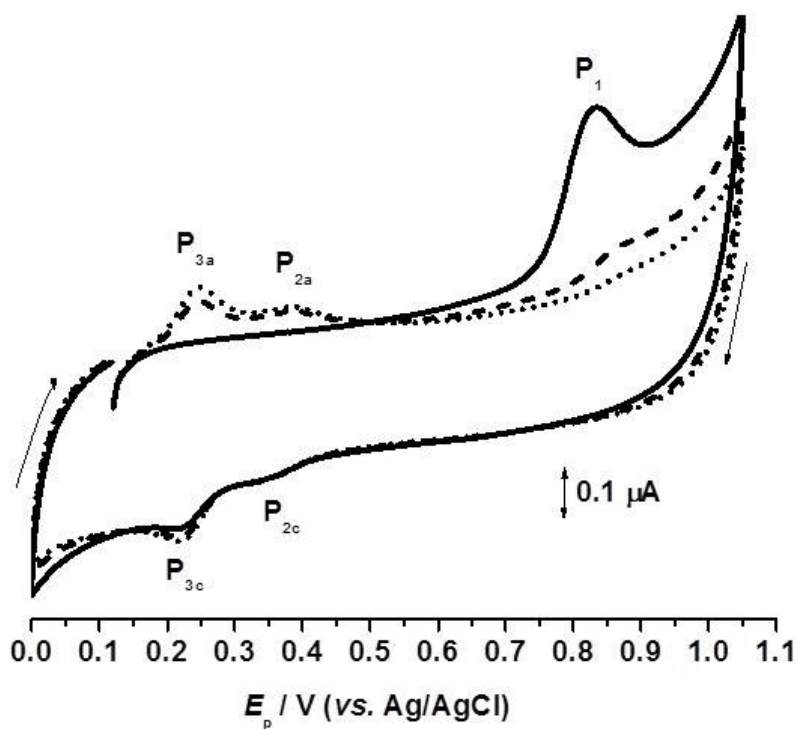


Fig. 1. CV in 10 μM compound 7, in acetate buffer pH 4.4, at GCE: (—) first, (---) fifth and (•••) tenth scan. Scan rate 50 $mV s^{-1}$.

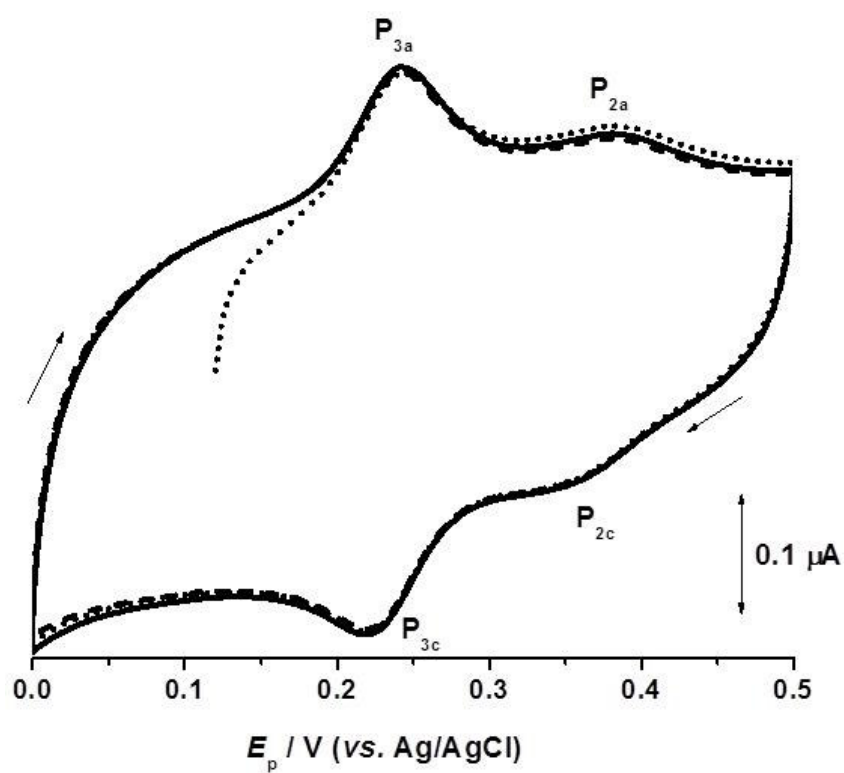


Fig. 2. CV of compound **7** adsorbed at the GCE surface, in acetate buffer pH 4.4: (•••) first, (—) fifth and (—•—) tenth scan after transfer to the buffer solution. Scan rate 50 mV s^{-1} .

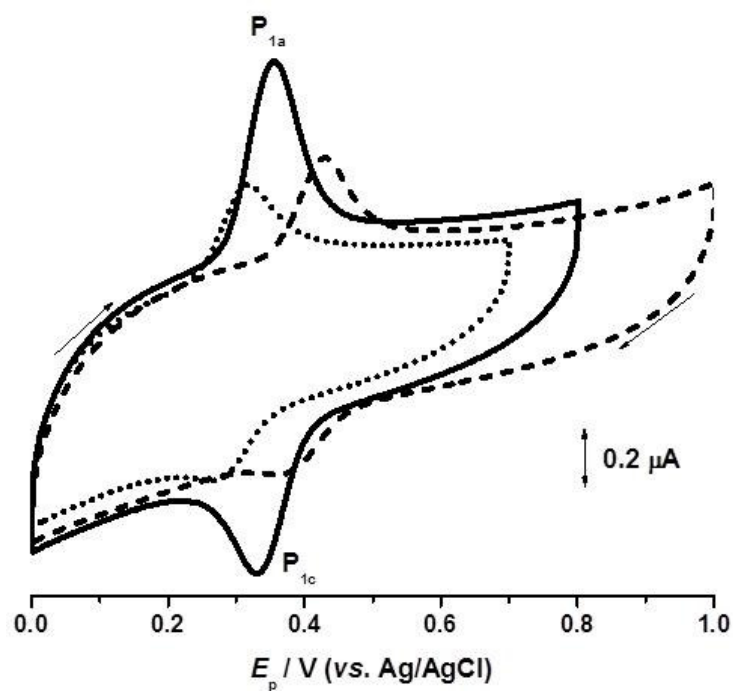


Fig. 3. CV in 10 μM compound **9**, first scan in acetate buffer, at GCE:

(---) pH 3.5, (—) pH 4.4 and (···) pH 5.3. Scan rate 50 mV s^{-1} .

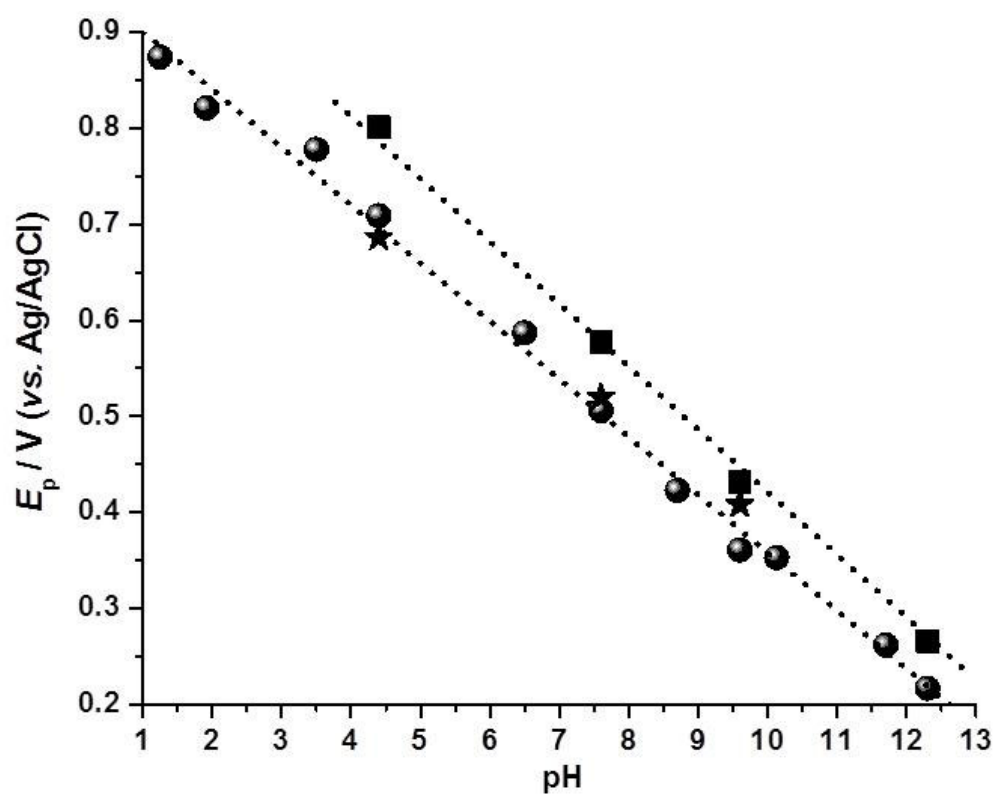


Fig. 4. Plot of E_{p1a} vs. pH of compounds **6** (●), **7** (■) and **8** (★).

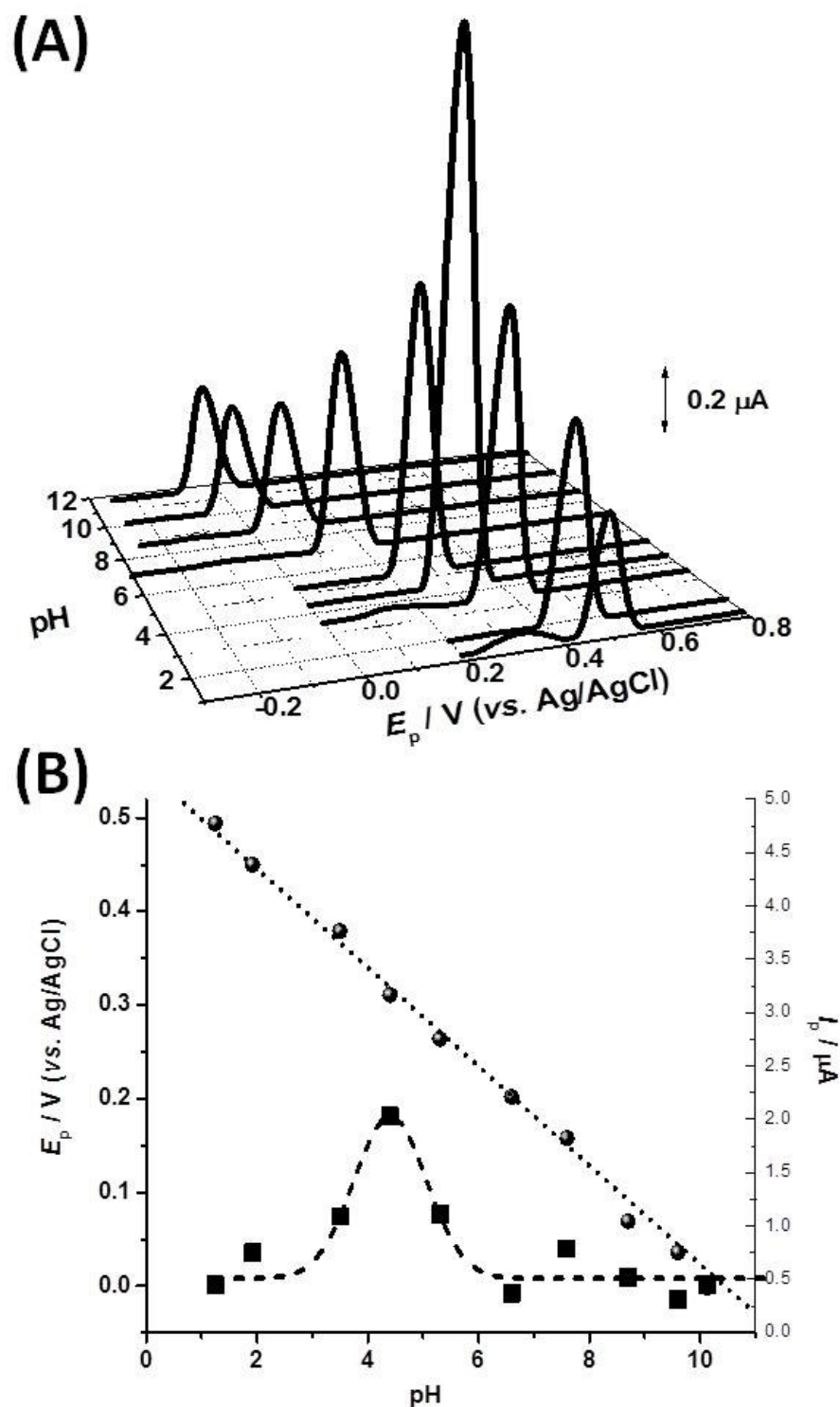


Fig. 5. (A) 3D plot of DP voltammograms baseline corrected in compound **9** vs. pH; (B) Plot of E_{p1a} (●), and I_{p1a} (■) vs. pH.

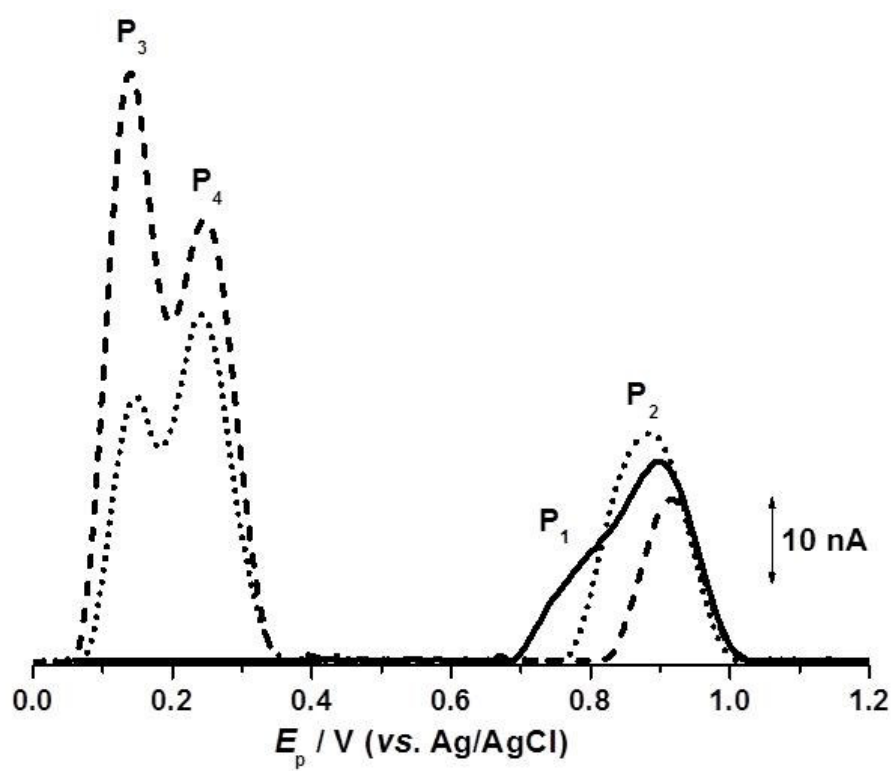


Fig. 6. DP voltammogram in 50 μ M compound **10**, in acetate buffer pH 3.5:

(—) first, (•••) second and (---) tenth scan. Scan rate 5 mV s^{-1} .

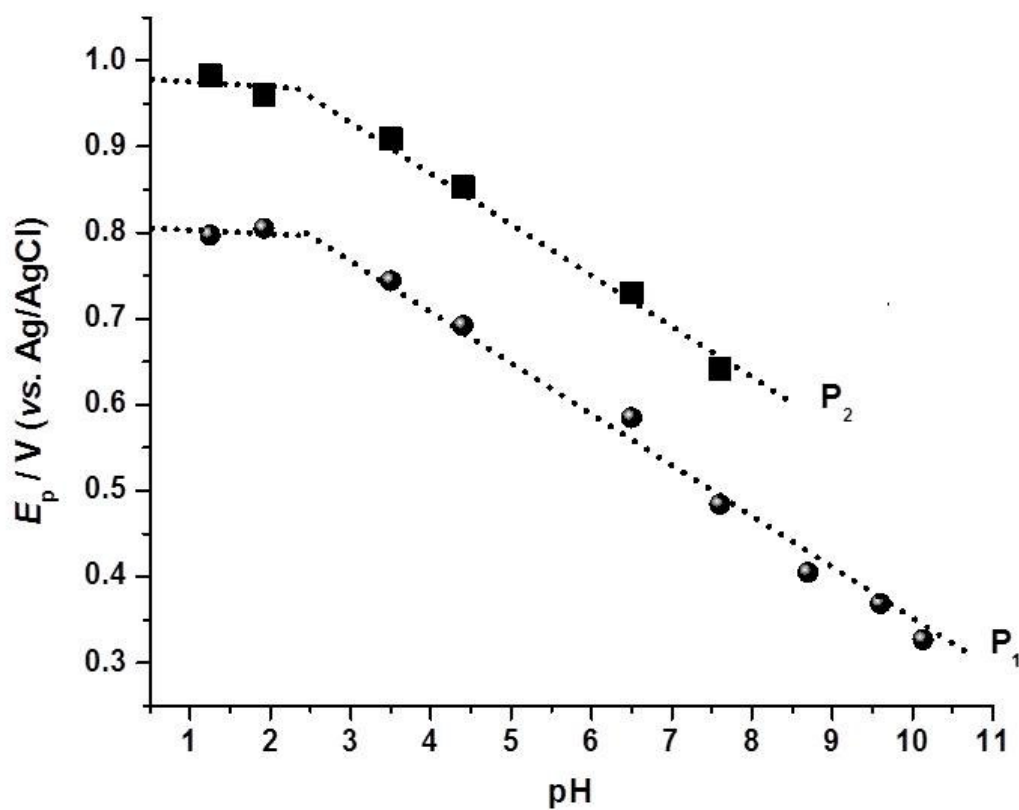


Fig. 7. Plot of E_{p1a} (●) and E_{p2a} (■) vs. pH of compound 10.

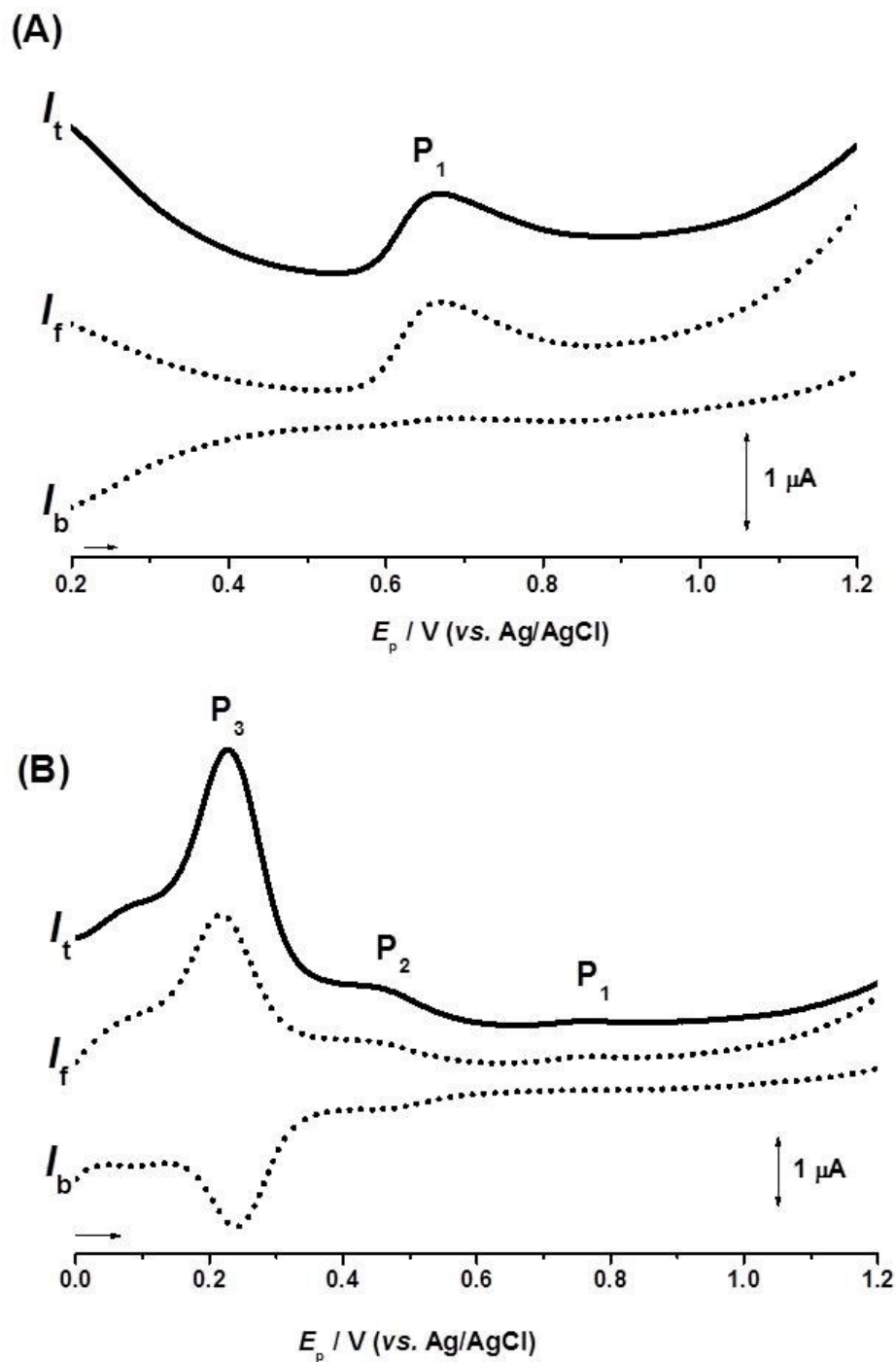


Fig. 8. SW voltammograms in 50 μM compound **8**, in acetate buffer pH 4.4:

(A) first and (B) third scan. I_t – total current, I_f – direct current and

I_b – forward current; $f = 25 \text{ Hz}$, $\Delta E = 2 \text{ mV}$, $v_{\text{eff}} = 50 \text{ mV s}^{-1}$.

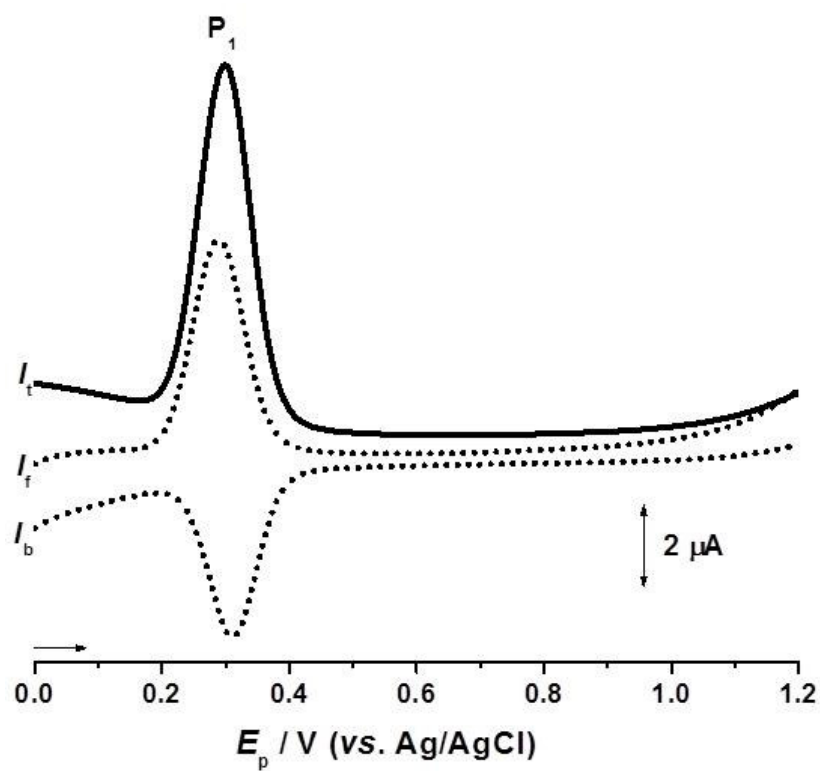


Fig. 9. SW voltammograms in 10 μM compound **9** third scan, in acetate buffer at pH 5.3:

I_t – total current, I_f – direct current and I_b – forward current; $f = 25$ Hz,

$$\Delta E = 2 \text{ mV}, v_{\text{eff}} = 50 \text{ mV s}^{-1}.$$

Synthesis and electrochemical study of new 3-(hydroxyphenyl)benzo[f]coumarins

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Highlights

- New hydroxyl substituted 3-arylbenzo[f]coumarins (compounds **6-10**) have been designed and synthesized.
- The hydroxyl substituted 3-arylbenzo[f]coumarins are compounds with important antioxidant properties,
- The hydroxyl substituted 3-arylbenzo[f]coumarins structure/electrochemical relationship was clarified.
- The electrochemistry oxidation investigated and the redox mechanisms proposed.